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The Effect of Melatonin Against FK506-Induced Renal Oxidative Stress in Rats

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Abstract

Background: Nephrotoxicity is an important side effect of FK506 and oxidative stress has been considered as one of the possible mechanisms. The present investigation examines the ability of melatonin to protect against FK506-induced renal oxidative stress. *Methods:* Thirty rats were divided into 3 groups (n = 10 each group). Group A was the sham group. Group B received 14 days FK506 (5 mg/kg/d, intraperitoneally [i.p.]) and group C received FK506 (5 mg/kg/d, i.p.) together with melatonin (4 mg/kg, i.p.) for 14 days. Kidney tissues were harvested to determine the tissue levels of malondialdehyde (MDA), total nitrite and nitric oxide (NO), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6). *Results:* In group C, the levels of TNF-α, IL-6, and NO were lower than in the group B ($P < .01$, $P < .03$, and $P < .04$, respectively) and although MDA levels were lower than in group B, the differences were not statistically significant ($P > .05$). *Conclusion:* These results suggest that melatonin has protective effect against FK506-induced renal oxidative stress.

Keywords

melatonin, tacrilimus, renal oxidative stress

Introduction

FK506 is a potent immunosuppressant used in solid organ transplantation and other immunologic diseases. FK-506 inhibits Ca^{2+}-dependent transcription of lymphokine genes in T-cells, and thereby acts as a powerful immunosuppressant.[1] However, its potential therapeutic applications may be seriously limited by several side effects, including nephrotoxicity and neurotoxicity.[1-3] There are many mediators associated with the pathogenesis of nephrotoxicity; however, the exact mechanism is still unclear.

FK-506 is a calcineurin inhibitor. The pivotal role of calcineurin activity in regulating intracellular calcium concentrations, the stimulatory effect of NF-κB on mammatory cytokine production, the influence of various growth factors on mesangial proliferation and interstitial fibrosis, and the disruption of several systems (NO, endothelin-1, thomboxane) regulating renal vascular tone are all important elements in determining the renal response to FK506 and similar agents.[1] Oxidative stress has been considered to be an important mediator of immunosuppressant-induced renal injury.[4,5] Free oxygen radicals formed within cells at oxidative stress can oxidize various molecules leading to cell death and tissue injury.[6]

Melatonin (*N*-acetyl-5-methoxytryptamine) is a highly lipophilic molecule secreted from the pineal gland. Melatonin, a compound with well-known antioxidant properties, directly scavenges a variety of toxic oxygen and nitrogen-based reactants and stimulates antioxidative enzymes.[7,8] It has been shown that melatonin plays a protective action against cyclosporine-induced oxidative stress and carbon tetrachloride induced hepatotoxicity oxidative stress by its antioxidant effects.[9,10] The aim of this study is to evaluate whether melatonin administration would protect against FK506-induced nephrotoxity in rats with its antioxidant effect. This effect of melatonin may be useful in lowering renal oxidative stress in patients with liver transplantation who has tacrolimus induced nephrotoxicity.

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Material and Method

Thirty 3 month-old male Swiss-albino rats weighing 300 to 350 g were included in the study. The study was approved by Inonu University Ethics Committee. All experiments were performed in accordance with the guidelines for animal research from the National Institutes of Health and were approved by our Committee on Animal Research. The animals were housed in stainless-steel cages under controlled temperature and humidity conditions and in a quiet room with a 12-hour/12-hour light/dark cycle. Rats were maintained on a standard laboratory diet with tap water ad libitum throughout the experiment, except for an overnight fast before surgery. All surgical procedures were performed under sterile conditions. In all, 30 rats were recruited in the study and divided into 3 groups (n = 10 each group). Group A was the sham group. Group B received 14 days FK506 (5 mg/kg/d, intraperitoneally [i.p.]) and Group C received FK506 (5 mg/kg/d i.p.) together with melatonin (4 mg/kg i.p.) 14 days. To assess effectiveness of melatonin against FK506 -induced nephrotoxicity in rats, we measured the activities of kidney tissues malondialdehyde (MDA), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), total nitrite and nitrate (NO).

For IL-6 and TNF-α levels, kidney tissue homogenates were prepared using a homogenizer IKA Ultra-Turnax (2 cycles of 45 seconds at 0°C) in 0.5 mol TRIS/1.5 mol NaCl/50 mmol $CaCl_2$/2 mmol sodium azide buffer at pH 7. The homogenates were then centrifuged at 10 000*g* for 15 minutes at a temperature of 4°C and the supernatants were used for enzyme-linked immunosorbent assay (ELISA). IL-6 and TNF-α (BioSource Immunoassay kit, Camarillo, CA) levels were measured using a sandwich ELISA protocol supplied by the manufacturer of the antibodies and resultant optical density determined using a microplate reader at 450 nm. Results are expressed as pg/g tissue.

For MDA and NO levels; kidney were homogenized in 1.15% KCl buffer (1:9 w/v) using a manual glass homogenizer (Tempest Virtishear, model 278069; The Virtis Company, Gardiner, NY) for approximately 5 minutes. NO is rapidly oxygenated to NO_2 and further to NO_3. As the direct assessment of NO is almost impossible (in vivo), the combined production of NO_2 and NO_3 can be used to assess NO in vitro and in vivo. Nitrate was assayed by a modification of the cadmium reduction method.[11] The produced nitrite was determined by diazotization of sulfanilamide and coupling to naphthylethylene diamine. After samples were deproteinized with somogyl reagent, the nitrate was reduced by Cu-coated Cd in glycine buffer at pH 9.7. The reduction followed pseudo-first-order reaction kinetics. The solutions were mixed, and then absorbances were read against the blank at 545 nm after 20 to 60 minutes. Results are expressed as μmol/mg tissue.

MDA in tissues was determined by the method of Uchiyama and Mihara.[12] A 3-mL aliquot of 1% phosphoric acid and 1 mL of 0.6% thiobarbituric acid solution were added to 0.5 mL of 10% tissue homogenate pipetted into a tube. The mixture was heated in boiling water for 45 minutes. After cooling, the color was extracted into 4 mL of *n*-butanol. The absorbance was measured in a spectrophotometer (Ultraspec Plus, Pharmacia LKB Biochrom, UK) at 532 nm. The amounts of lipid peroxides were calculated as thiobarbituric acid reactive substances of lipid peroxidation and are given as nmol/g tissue.

Statistical Analysis

The statistical package for social sciences (SPSS) version 13.00 was used for the statistical analysis. Individual group parameters were assessed using the Mann–Whitney *U* test. The results are shown in the text as mean values ± standard deviations for all comparisons with significance defined as $P < .05$.

Results

All the animals survived until the end of the experiment. The results of MDA and NO are shown in Table 1. Although the MDA levels were lower in the melatonin-treated rats group than in the FK506 group, the differences were not statistically significant ($P > .05$). In the melatonin-treated rats, the levels of NO were lower than in the FK506 group ($P < .04$). In the melatonin-treated rats, the levels of TNF-α and IL-6 were lower than in the FK506 group ($P < .01$ and $P < .03$, respectively).

Discussion

Immunosuppression-induced nephrotoxicity remains an unavoidable and significant problem in organ transplantation. Immunosuppressive agents such as FK506 have been shown to promote the generation of reactive oxygen species in a number of cell types.[5] Long-term treatment with FK506 is associated with nephrotoxicity.[13] The mechanisms of this toxicity are not fully understood, but they seem to be associated with increased production of oxygen free radicals. Although there are many parameters and potential mechanisms of nephrotoxicity, this study was limited to oxidative stress parameters.

Although melatonin is known to have antioxidant properties, to date, it has not been investigated in

Table 1. Tissue Levels of MDA, NO, TNF-α, and IL-6 Activities in the Groups[a]

Groups	MDA (nmol/g tissue)	NO (μmol/mg tissue)	TNF-α (pg/g tissue)	IL-6 (pg/g tissue)
I: Group A (n = 10)	33.8 ± 2.1	61.8 ± 3.4	3.6 ± 0.2	1.6 ± 0.9
II: Group B (n =10)	46.8 ± 6.2	91.5 ± 4.5	5.3 ± 0.8	2.3 ± 0.2
III: Group C (n = 10)	42.1 ± 4.9	82.4 ± 10.2	4.3 ± 0.3	2.0 ± 0.2
P values[b]				
I vs II	.001	.001	.001	.001
II vs III	.06	.04	.01	.03

Note: MDA= malondialdehyde; NO= total nitrite and nitrate; TNF-a= tumor necrosis factor-alpha; IL-6= interleukin-6.
[a]Group A = sham; group B = FK506, group C = FK506 + melatonin.
[b]$P < .05$ was considered to be statically significant.

experimental FK506-induced renal oxidative stress in rats. The present study demonstrates that intraperitoneal administration of melatonin at a dose of 4 mg/kg/d over 14 days caused a decrease in MDA and NO values. MDA is a secondary product of oxidative stres formed during lipid peroxidation, and it is released as a result of the toxic effect of reactive oxygen species in rats after drug-induced nephrotoxicity.[13] Increased concentrations of MDA reflect the level of lipid peroxidation in tissues and are considered a marker of tissue injury.[14] There are several reports indicating that levels of MDA increase in FK506-induced nephrotoxicity in rats.[15] Our results are consistent with previous reports of high levels of MDA.

Levels of MDA in melatonin-treated rat kidney tissue were found to be lower than in the FK506 group in this study. Although tissue MDA levels were clearly decreased by melatonin, the mechanism remains unclear. Because indirect antioxidant and direct free radical scavenger properties of melatonin are known,[16,17] reductions in MDA levels in the melatonin-treated rats were likely to be due to these antioxidant and free radical scavenging effects. Melatonin is readily absorbed when administered via the intraperitoneal route and it prevents oxidative damage, preserves mitochondrial function,[18] and has low toxicity. It is possible that the interference of melatonin with free radical generation may be related to a decline in renal oxidative stress.

NO is a free radical synthesized from L-arginine by nitric oxide synthase (NOS) in biological systems.[19] It has been suggested that NO, in particular, generated via the induction of the NOS2 enzyme isoform, is an important cytotoxic contributor in the process of allograft rejection.[20] In the present study, the levels of NO in melatonin-treated rats were significantly lower than in the FK506 group. Watari et al[21] showed that FK506 did not change the level of serum $NO_2 + NO_3$, compared with the vehicle group. However, in our study, serum levels of $NO_2 + NO_3$ were different in each group. This may be because of the different doses of FK506 given to rats in our study. Watari et al[21] administered FK506 to rats intraperitonealy at dosages of 0.1 to 2.0 mg/kg/h over 60 minutes, whereas we administered 5 mg/kg/d for 14 days. Because melatonin has a decreasing effect on NO, the use of melatonin with FK506 may decrease oxidative stress–related nephrotoxicity in renal or liver transplant patients.

Several mechanisms may contribute to the reduced levels of NO in melatonin-injected rats. For example, melatonin may be capable of reducing peroxynitrite generation because of its ability to scavenge NO[22] and to inhibit one isoform of NOS.[23] Also, recent reports have shown that melatonin directly neutralizes ONOO.[24] Serum nitrite and nitrate levels rise in the acute phase of rejection of a vascularized allograft.[20,25] Moreover, FK506 and cyclosporine A inhibit graft rejection and the latter has been shown to prevent the elevation of serum nitrite and nitrate, which has been attributed to inhibition of cytokine production by T cells by this immunosuppressant.[20]

Free radicals and cytokines can cause cellular injury. It has been reported that nephrotoxicity affected several parameters, such as expression of various pro-inflammatory genes, including NOS, TNF-α, and IL-6. FK506 induces IL-6 production through the activation of transcription factor nuclear factor (NF)-κB. IL-6 has been shown to produce renal abnormalities in vivo, such as mesangioproliferative glomerulonephritis.[26]

FK506 reduced IL-1 and IL-2 production, both of which increase free radical scavenging enzymes. On this basis, it might be expected that enzymes such as glutathione peroxidase, glutathione reductase, and superoxide dismutase would be reduced.[1]

Active macrophages and monocytes are primary sources of TNF in pathological conditions. Pro-inflammatory agents found in plasma, such as complement factors, cytokines (IL-6, IL-8, TNF), platelet-activating factor, and leukotrienes, can also cause endothelial damage. As a T cell blocker, FK506 exhibits an immunosuppressive effect and inhibits cytokine secretion by T cells. It is considered that FK506 modulates neutrophil infiltration by its effects on cytokine and lymphokine secretion.[27-30]

TNF-α is established as a major mediator of renal ischemia/reperfusion injury and has been associated

with endotoxemia. Indeed, the inflammatory response is orchestrated by cytokines and TNF-α is one of the major pro-inflammatory cytokines. Oxidative stress could also be correlated with the induction of a pro-inflammatory cascade mediated by TNF-α.[31]

The lack of information on FK506 levels is a limitation of this study, because all the differences observed may relate to melatonin effects on FK506 metabolism, as many drug effects are related to the effects of P450 metabolism of the drug. We did not study renal function in the rats, FK506 levels, or conduct a histopathological examination of renal tissue.

In conclusion, renal oxidative stress was observed in FK506 (5 mg/kg/d for 14 days) administered rats. The present study demonstrated that intraperitoneal administration of melatonin reduced FK506-induced kidney oxidative damage. Further investigations are required to evaluate the protective effect of melatonin in FK506-induced kidney oxidative damage in experimental and clinical models.

Declaration of Conflicting Interests

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References

1. Dumont F, Staruch MJ, Koprak SL, Sierkaka JJ. The immunosuppressive and toxic effects of FK-506 are mechanistically related: pharmacology of a novel antagonist of FK-506 and rapamycin. *J Exp Med*. 1992;176:751-760.
2. Finn WF. FK506 nephrotoxicity. *Ren Fail*. 1999;21: 319-329.
3. Yamauchi A, Oishi R, Kataoka Y. Tacrolimus-induced neurotoxicity and nephrotoxicity is ameliorated by administration in the dark phase in rats. *Cell Mol Neurobiol*. 2004;24:695-704.
4. Han SY, Mun KC, Choi HJ, et al. Effects of cyclosporine and tacrolimus on the oxidative stress in cultured mesangial cells. *Transplant Proc.* 2006;38:2240-2241.
5. Zhou X, Yang G, Davis CA, et al. Hydrogen peroxide mediates FK506-induced cytotoxicity in renal cells. *Kidney Int*. 2004;65:139-147.
6. Kim YS, Jhon DY, Lee KY. Involvement of ROS and JNK1 in selenite-induced apoptosis in Chang liver cells. *Exp Mol Med*. 2004;36:157-164.
7. Reiter RJ, Tan DX, Mayo JC, Sainz RM, Leon J, Czarnocki Z. Melatonin as an antioxidant biochemical mechanism and pathophysiological implications in humans. *Acta Biochim Pol*. 2003;50:1129-1146.
8. Reiter RJ, Carneiro RC, Oh CS. Melatonin in relation to cellular antioxidative defense mechanism. *Horm Metab Res*. 1997;29:363-372.
9. Rezzani R, Buffoli B, Rodella L, Stacchiotti A, Bianchi R. Protective role of melatonin in cyclosporine A-induced oxidative stress in rat liver. *Int Immunopharmacol*. 2005;5:1397-1405.
10. Kus I, Ogeturk M, Oner H, Sahin S, Yekeler H, Sarsilmaz M. Protective effects of melatonin against carbon tetrachloride-induced hepatotoxicity in rats: a light microscopic and biochemical study. *Cell Biochem Funct*. 2005;23:69-74.
11. Grisotto PC, dos Sandos AC, Continho-Netto J, Cherri J, Piccinato CE. Indicators of oxidative injury and alterations of the cell membrane in the skeletal muscle of rats submitted to ischemia and reperfusion. *J Surg Res*. 2000;92:1-6.
12. Uchiama A, Mihara B. The biological significance of Malondialdehyde determination in the assesment of tissue oxidative stress. *Life Scien.* 1990; 48: 301-309.
13. Randhawa PS, Shapiro R, Jordan ML, Starzl TE, Demetris AJ. The histopathological changes associated with allograft rejection and drug toxicity in renal transplant recipients maintained on FK 506. Clinical significance and comparison with cyclosporine. *Am J Surg Pathol*. 1993; 17:60-68.
14. Ara C, Kirimlioglu H, Karabulut AB, et al. Protective effect of resveratrol against oxidative stress in cholestasis. *J Surg Res*. 2005;127:112-117.
15. Ahmed SS, Strobel HW, Napoli KL, Gravel J. Adrenochrome reaction implicates oxygen radicals in metabolism of cyclosporine A and FK-506 in rat and human liver microsomes. *J Pharmacol Exp Ther*. 1993;265:1047-1054.
16. Allegra M, Reiter RJ, Tan DX, Gentile C, Tesoriere L, Livrea MA. The chemistry of melatonin's interaction with reactive species. *J Pineal Res*. 2003;34:1-10.
17. Ara C, Kirimlioglu H, Karabulut AB, et al. Protective effect of melatonin against oxidative stress on adhesion formation in the rat cecum and uterine horn model. *Life Sci*. 2005;77:1341-1350.
18. Leon J, Acuna-Castroviejo D, Sainz RM, Mayo JC, Tan DX, Reiter RJ. Melatonin and mitochondrial function. *Life Sci*. 2004;75:265-290.
19. Palmer RM, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature*. 1988;333:664-666.
20. Winlaw DS, Schyvens CG, Smythe GA, et al. Urinary nitrate excretion is a noninvasive indicator of acute cardiac allograft rejection and nitric oxide production in the rat. *Transplantation*. 1994;58:1031-1036.
21. Watarai Y, Morita K, Shimoda N, et al. Effect of tacrolimus and cyclosporine on renal microcirculation and nitric oxide production. *Transplant Proc*. 2004;36:2130-2132.

22. Noda Y, Mori A, Liburdy R, Packer L. Melatonin and its precursors scavenge nitric oxide. *J Pineal Res*. 1999;27:159-163.
23. Pozo D, Reiter RJ, Calvo JR, Guerrero JM. Physiological concentrations of melatonin inhibit nitric oxide synthase in rat cerebellum. *Life Sci*. 1994;55:455-460.
24. Gilad E, Cuzzocrea S, Zingarelli B, Salzman AL, Szabó C. Melatonin is a scavenger of peroxynitrite. *Life Sci*. 1997;60:PL169-PL174.
25. Langrehr JM, Murase N, Markus PM, et al. Nitric oxide production in host-versus-graft and graft-versus-host reactions in the rat. *J Clin Invest*. 1992;90:679-683.
26. Muraoka K, Fujimoto K, Sun X, et al. Immunosuppressant FK506 induces interleukin-6 production through the activation of transcription factor nuclear factor (NF)-kB: implications for FK506 nephropathy. *J Clin Invest*. 1996;97:2433-2439.
27. Graeme MR. Ischaemia/reperfusion, inflammatory responses and acute lung injury. *Thorax*. 1997;52:841-842.
28. Tilney NL, Paz D, Ames J, Gasser M, Laskowski I, Hancock WW. Ischemia-reperfusion injury. *Transplant Proc*. 2001;33:843-844.
29. Kubes P, Hunter J, Granger DN. Effects of cyclosporine A and FK506 on ischemia reperfusion injury induced neutrophil infiltration in the cat. *Dig Dis Sci*. 1991;36: 1469-1472.
30. Reilly PM, Schiller HJ, Bulkley GB. Pharmacologic approach to tissue injury mediated by free radicals and other reactive oxygen metabolites. *Am J Surg*. 1991;161:488-503.
31. Akbulut G, Dilek ON, Kahraman A, Köken T, Serkeser M. The correlation between renal tissue oxidative stress parameters and TNF-α levels in an experimental model of ischemia-reperfusion injury in mice. *Turk J Trauma Emerg Surg*. 2005; 11:11-16.